



**UNIVERSITI PUTRA MALAYSIA**

**STUDIES ON THE CHARACTERISATION AND UTILISATION OF A  
NEW PHYTASE-PRODUCING BACTERIUM ISOLATED  
FROM THE RUMEN OF CATTLE**

**LAN GANQIU**

**IB 2001 4**

**STUDIES ON THE CHARACTERISATION AND UTILISATION OF A  
NEW PHYTASE-PRODUCING BACTERIUM ISOLATED  
FROM THE RUMEN OF CATTLE**

**By**

**LAN GANQIU**

**Thesis Submitted in Fulfilment of the Requirements for the Degree of  
Doctor of Philosophy in the Institute of Bioscience  
Universiti Putra Malaysia**

**September 2001**



**This thesis is dedicated to  
my late parents, my loving wife, Xiao Lin and my daughter, Di Yi.**

Abstract of the thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

**STUDIES ON THE CHARACTERISATION AND UTILISATION OF A  
NEW PHYTASE-PRODUCING BACTERIUM ISOLATED  
FROM THE RUMEN OF CATTLE**

**By**

**LAN GANQIU**

**September 2001**

**Chairman: Professor Tan Sri Dato' Dr. Syed Jalaludin bin Syed Salim**  
**Faculty: Institute of Bioscience**

Five phytase-producing bacterial strains isolated from the rumen of cattle were identified to be a new bacterial species based on their morphological, physiological, biochemical and molecular characters. The new species is named *Mitsuokella jalaludinii*. *Mitsuokella jalaludinii* hydrolysed sodium phytate rapidly and the phytase production was strongly induced by phytate present in the medium. Rice bran (RB) and soybean milk (SM) were found to be the best carbon and nitrogen sources, respectively, for phytase production by *M. jalaludinii*. Phosphate at a level of 0.05 – 0.5% in RB-SM medium had no effect on phytase production. Glucose added to RB-SM medium had a negative effect on phytase production of *M. jalaludinii*. The optimum temperature and optimum initial pH for phytase production of *M. jalaludinii* were 39 °C and about 7.0, respectively.

The activity of *M. jalaludinii* phytase was highest at 55 – 60 °C and pH 4.0 – 5.0. It was specific to phytate as a substrate, significantly stimulated by  $\text{Ba}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Ca}^{2+}$  and significantly inhibited by  $\text{Zn}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$  and  $\text{Fe}^{3+}$ . The metal ion chelators and phosphate were not the inhibitors of *M. jalaludinii* phytase activity.

Acute pathogenicity tests indicated that *M. jalaludinii* was non-pathogenic to chickens and mice. Supplementation of *M. jalaludinii* culture to corn soybean meal feed for chickens significantly increased P released from the feed *in vitro* and P, DM and CP digestibilities *in vivo*. About 70% of *in vivo* response in P digestibility and 90% of *in vivo* response in DM and CP digestibilities or AME value could be predicted by the P released *in vitro*. *Mitsuokella jalaludinii* phytase was most active in the crop of broiler chickens and was inactivated in the stomach.

Supplementation of either fresh active *M. jalaludinii* culture (AMJC) or freeze-dried active *M. jalaludinii* culture (FD-AMJC) or Natuphos<sup>®</sup> phytase to low-aP diet significantly improved the feed intake, body weight gain and feed conversion ratio of broilers. The digestibilities of DM, CP, P, Ca, and Cu and the AME value of diet were significantly increased by the supplementation of AMJC. Supplementation of AMJC or FD-AMJC or Natuphos<sup>®</sup> phytase to low-aP diet significantly increased the tibia ash content and serum P concentration but significantly reduced Mn concentration in tibia ash of broiler chickens. Chicks receiving FD-AMJC had better ( $P<0.05$ ) feed conversion rate as compared to those receiving Natuphos<sup>®</sup> phytase. FD-AMJC supplementation to low-aP diet significantly ( $P<0.05$ ) increased the AME value of diet and the digestibilities of DM, CP, P, Ca and Cu (11 to 13-day-old and 18 to 20-day-old chicks) but Natuphos<sup>®</sup> phytase supplementation only significantly improved the digestibilities of DM, P (11 to 13-day-old and 18 to 20-day-old chicks) and Ca (11 to 13-day-old chicks). Chicks receiving low-aP diet added with AMJC or FD-AMJC or Natuphos<sup>®</sup> phytase had similar ( $P>0.05$ ) performance as those receiving normal-aP diet but excreted less ( $P<0.05$ ) phosphorus.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**KAJIAN MENGENAI PENCIRIAN DAN PENGGUNAAN  
SPESIS BAKTERIA BARU PENGHASIL FITASE  
DARI RUMEN LEMBU**

**Oleh**

**LAN GANQIU**

**September 2001**

**Pengerusi: Professor Tan Sri Dato' Dr. Syed Jalaludin bin Syed Salim  
Fakulti: Institut Biosains**

Lima strain bakteria yang menghasilkan fitase telah dipencil dan dikenalpasti sebagai satu spesis baru berdasarkan ciri-ciri morfologi, fisiokimia dan molekul. Spesis ini dinamakan *Mitsuokella jalaludinii*. Bakteria ini menghidrolisis sodium fitate dan penghasilan fitase diaruhi oleh fitate yang terdapat di dalam medium pertumbuhan. Dedak padi (RB) dan susu kacang soya (SB) merupakan sumber utama karbon dan nitrogen. Penambahan glukosa pada medium RB-SB tidak memberikan kesan positif dalam penghasilan fitase. Suhu optimum penghasilan fitase adalah 39°C dan pH optimum, 7.0. Aktiviti fitase paling tinggi pada suhu 55 - 60°C dan pada pH 4-5. Aktivitinya spesifik terhadap fitat sebagai substrat, dirangsang oleh Ba<sup>2+</sup>, Mn<sup>2+</sup> dan Ca<sup>2+</sup>, dan dihalang oleh Zn<sup>2+</sup>, Cu<sup>2+</sup>, Fe<sup>2+</sup> dan Fe<sup>3+</sup>. Ion galian dan P didapati tidak merencat aktiviti fitase.

Ujian patogenisiti akut menunjukkan *Mitsuokella jalaludinii* tidak patogenik terhadap ayam dan tikus. Penambahan kultur *Mitsuokella jalaludinii* ke dalam makanan ayam yang mengandungi jagung dan kacang soya, meningkatkan pembebasan P secara *in vitro*, dan meningkatkan penghadaman P, DM dan CP

secara *in vivo*. Lebih kurang 70% penghadaman P *in vivo* dan 90% penghadaman DM dan CP *in vitro* atau nilai AME boleh diramalkan dari P yang dibebaskan secara *in vitro*. Fitase yang dihasilkan oleh *Mitsuokella jalaludinii* paling aktif pada tembolok ayam tetapi tidak aktif di bahagian perut.

Penambahan kultur *Mitsuokella jalaludinii* dalam bentuk kultur aktif-segar (AMJC) dan kultur aktif-kering-beku (FD-AMJC) serta 'Natuphos<sup>®</sup> phytase' ke diet rendah-fosfat meningkatkan pengambilan makanan, penambahan berat badan dan nisbah penukaran makanan ayam. Penghadaman DM, CP, P, Ca dan Cu serta nilai AME diet meningkat ( $P < 0.05$ ) dengan penambahan AMJC. Penambahan samada AMJC, FD-AMJC atau 'Natuphos<sup>®</sup> phytase' pada diet rendah-fosfat, meningkatkan kandungan abu tibia dan P serum tetapi mengurangkan Mn abu tibia ayam. Ayam yang di beri penambahan FD-AMJC menunjukkan nisbah penukaran makanan yang lebih baik daripada ayam yang di beri penambahan Natuphos<sup>®</sup> phytase. Penambahan FD-AMJC pada diet rendah-fosfat meningkatkan ( $P < 0.05$ ) nilai AME serta penghadaman DM, CP, P, Ca dan Cu (pada ayam umur 11–13 hari dan 18 –20 hari) tetapi penambahan 'Natuphos<sup>®</sup> phytase' hanya meningkatkan penghadaman DM, P (pada ayam umur 11– 13 hari dan 18 – 20 hari) dan Ca (pada ayam umur 11– 13 hari). Ayam yang diberi diet rendah-fosfat yang ditambah AMJC atau FD-AMJC atau 'Natuphos<sup>®</sup> phytase' menunjukkan prestasi yang sama ( $P > 0.05$ ) dengan ayam yang di beri diet normal-fosfat tetapi P kurang ( $P < 0.05$ ) dikeluarkan.

## ACKNOWLEDGEMENTS

I wish to express my deep appreciation and most sincere gratitude to the chairman of the supervisory committee, Professor Tan Sri Dato' Dr. Syed Jalaludin bin Syed Salim, (Vice-Chancellor of Universiti Putra Malaysia until his retirement on 16 April 2001), for his invaluable guidance and advice throughout the course of my study in Malaysia.

I am deeply grateful and indebted to Professor Dr. Ho Yin Wan, a supervisory committee member and Deputy Director of the Institute of Bioscience, for her kind assistance, advice, endless support and encouragement throughout the duration of this study and for her critical comments and constructive suggestions during the preparation of my thesis.

I also wish to express my deep appreciation and sincere gratitude to Associate Professor, Dr. Norhani Abdullah, another supervisory committee member, at the Department of Microbiology and Biochemistry, Faculty of Science and Environmental Study, for her advice, guidance, and helpful suggestions, throughout the course of my work.

I am indebted to Madam Haw Ah Kam, Mr. Khairul Kamar Bakri, Mr. Jivananthan Arumugam, Mr. Nagayah Muniandy and Mr. Paimon Lugiman, staff of Digestive Microbiology Research Centre; Mr. Ibrahim Mohsin, staff of Animal Nutrition Laboratory; and Miss Suleka Madhavan, Mr. Ho Oi Kuan and Miss Azilah Abdullah Jalil, staff of Electron Microscopy Unit, for their technical assistance and co-operation.



I also wish to thank Associate Professor Dr. Hair-Bejo, Department of Pathology, Faculty of Veterinary, for his helpful advice in the pathogenicity study.

I wish to extend my sincere thanks to Chin Chin, Kala, Darlis, Thongsuk, Latiffah, Lee, Wan, Vicky and Sidig, my post-graduate friends in the Digestive Microbiology Research Centre, who have assisted me by providing invaluable ideas and support which contributed to the successful accomplishment of this work. Special thanks are also due to my friend and former classmate, Dr. Jin Lizhi, for his help, support and encouragement during my study in Malaysia.

I would also like to thank Universiti Putra Malaysia and the Malaysian government for the scholarship which enables me to pursue my PhD degree.

Finally, very special thanks are extended to my loving wife, Xiao Lin, for her sacrifice, patience, understanding, support and encouragement throughout the study. Her love and support have made all this come true.

I certify that an Examination Committee met on 24<sup>th</sup> September 2001 to conduct the final examination of Lan Ganqiu on his Doctor of Philosophy thesis entitled “Studies on the Characterisation and Utilisation of a New Phytase-producing Bacterium Isolated from the Rumen of Cattle” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulation 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**ABDUL RAZAK BIN ALIMON, Ph.D.**

Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**SYED JALALUDIN BIN SYED SALIM, Ph.D.**

Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Member)

**HO YIN WAN, Ph.D.**

Professor  
Institute of Bioscience  
Universiti Putra Malaysia  
(Member)

**NORHANI BT. ABDULLAH, Ph.D.**

Associate Professor  
Faculty of Science and Environmental Studies  
Universiti Putra Malaysia  
(Member)

**RYOJI ONODERA, Ph.D.**

Professor  
Division of Animal Science  
Faculty of Agriculture  
Miyazaki University  
Japan  
(Independent Examiner)



---

**MOHD GHAZALI MOHAYIDIN, Ph.D.**  
Professor / Deputy Dean of Graduate School  
Universiti Putra Malaysia

Date: **29 SEP 2001**

This thesis submitted to the Senate of Universiti Putra Malaysia has been accepted as fulfilment of the requirements for the degree of Doctor of Philosophy.



---

**AINI IDERIS, Ph.D.**  
Professor / Dean of Graduate School  
Universiti Putra Malaysia

Date: **08 NOV 2001**

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutes.



---

LAN GANQIU

Date: 28 September 2001

## TABLE OF CONTENTS

	Page
DEDICATION.....	ii
ABSTRACT.....	iii
ABSTRAK.....	v
ACKNOWLEDGEMENTS.....	vii
APPROVAL SHEETS.....	ix
DECLARATION FORM.....	xi
LIST OF TABLES.....	xvi
LIST OF FIGURES.....	xxii
LIST OF ABBREVIATIONS.....	xxv
CHAPTER	
1 INTRODUCTION.....	1
2 LITERATURE REVIEW.....	6
2.1 Poultry Industry and Its Environmental Challenges.....	6
2.2 The Nutritional Importance of Phosphorus .....	7
2.3 Phosphorus and Environmental Pollution.....	8
2.4 Source of Phosphorus and Its Biological Effectiveness for Poultry..	
2.5 The Occurrence and Distribution of Phytic Acid.....	10
2.6 The Chemical Structure and Composition of Phytic Acid.....	12
2.7 Interactions Between Phytate and Nutritional Components of Feed Ingredients.....	13
2.7.1 Phytate and Protein Interactions.....	14
2.7.2 Phytate and Mineral Interactions.....	15
2.8 Bioavailability of Phytate Phosphorus for Poultry.....	16
2.9 Effects of Phytate on Other Minerals Bioavailability.....	18
2.9.1 Calcium.....	19
2.9.2 Zinc.....	20
2.9.3 Other Mineral Elements.....	20
2.10 Sources and Purification of Phytase.....	21
2.10.1 Phytase from Plant.....	22
2.10.2 Phytase from Microorganisms.....	23
2.10.3 Phytase from Animal Tissues.....	25
2.11 Physicochemical and Catalytic Characteristics of Phytase.....	26
2.11.1 Molecular Weight, Optimum Temperature and Optimum pH.....	26
2.11.2 Kinetics.....	27
2.11.3 Substrate Selectivity.....	27
2.11.4 Activation and Inhibition.....	29
2.12 Application of Microbial Phytase to Improve the Availability of Nutrients for Poultry.....	33



	<b>Page</b>
2.12.1 Effects of Phytase on Phytate Phosphorus Utilisation and Performance of Poultry .....	33
2.12.2 Effectiveness of Phytase on the Availability of Other Mineral Nutrients.....	37
2.12.3 Effects of Phytase on Protein and Amino Acid Utilisation in Poultry.....	40
2.12.4 Factors Influencing the Efficacy of Microbial Phytase.....	41
 3 ISOLATION AND IDENTIFICATION OF PHYTASE- PRODUCING BACTERIA FROM THE RUMEN OF CATTLE.....	 45
3.1 Introduction.....	45
3.2 Materials and Methods.....	46
3.2.1 Animal and Diet.....	46
3.2.2 Anaerobic Technique.....	46
3.2.3 Preparation of Media.....	47
3.2.4 Collection and Preparation of Rumen Sample.....	49
3.2.5 Screening for Phytase-producing Rumen Bacterial Isolates.....	50
3.2.6 Determination of Phytase Activity of Rumen Sample.....	51
3.2.7 Identification of Phytase-producing Bacterial Isolates from the Rumen.....	52
3.3 Results.....	59
3.3.1 Ruminal Phytase Activity.....	59
3.3.2 Rumen Bacterial Strains that Hydrolysed Phytate...	60
3.3.3 Identification of the Phytase-producing Bacteria from Rumen.....	61
3.4 Discussion.....	74
 4 HYDROLYSIS OF SODIUM PHYTATE BY <i>MITSUOKELLA JALALUDINII</i> AND FERMENTATION CONDITIONS INFLUENCING ITS PHYTASE PRODUCTION.....	 79
4.1 Introduction.....	79
4.2 Materials and Methods.....	81
4.2.1 Determination of Phytate Hydrolysis by <i>M.</i> <i>jalaludinii</i> under Aerobic and Anaerobic Conditions.....	81
4.2.2 Phytase Induction Study.....	83
4.2.3 Fermentation Conditions Regulating Phytase Production.....	85
4.2.4 Optimisation of Rice Bran (RB) and Soybean Milk (SM) Concentration for Phytase Production.....	90
4.2.5 Statistical Analysis.....	91
4.3 Results .....	91

	Page
4.3.1 Growth of <i>M. jalaludinii</i> and Na-phytate Hydrolysis under Aerobic and Anaerobic Conditions.....	91
4.3.2 Phytase Induction Study.....	93
4.3.3 Fermentation Conditions Influencing Phytase Production of <i>M. jalaludinii</i> .....	99
4.3.4 Effects of Rice Bran (RB) and Soybean Milk (SM) Concentrations in RB-SM Medium on Growth and Phytase Activity of <i>M. jalaludinii</i> .....	108
4.4 Discussion.....	111
 5 PRELIMINARY PURIFICATION AND CHARACTERISATION OF PHYTASE FROM <i>MITSUOKELLA JALALUDINII</i> .....	 119
5.1 Introduction.....	119
5.2 Materials and Methods.....	121
5.2.1 Culture Conditions and Sample Preparation.....	121
5.2.2 Partial Purification of Phytase .....	121
5.2.3 Phytase and Protein Assay.....	123
5.2.4 Characterisation of Phytase Activity.....	124
5.2.5 Distribution of Enzymes.....	129
5.2.6 Statistical Analysis.....	130
5.3 Results.....	131
5.3.1 Purification of Phytase.....	131
5.3.2 Characterisation of Phytase from <i>M. jalaludinii</i> .....	133
5.3.3 Localisation of Phytase of <i>M. jalaludinii</i> .....	141
5.4 Discussion.....	143
 6 PATHOGENICITY TESTS OF <i>M. JALALUDINII</i> : ACUTE STUDIES IN MICE AND BROILER CHICKENS.....	 148
6.1 Introduction.....	148
6.2 Materials and Methods.....	148
6.2.1 Pathogenicity Test in Mice.....	148
6.2.2 Pathogenicity Test in Broiler Chickens.....	152
6.2.3 Statistical Analysis.....	154
6.3 Results.....	154
6.3.1 Pathogenicity Test in Mice.....	154
6.3.2 Pathogenicity Test in Chickens.....	160
6.4 Discussion.....	167
 7 <i>IN VITRO</i> AND <i>IN VIVO</i> STUDIES OF ENZYMATIC DEPHOSPHORYLATION OF PHYTATE IN CORN-SOYBEAN MEAL DIETS FOR BROILER CHICKENS BY PHYTASE OF <i>M. JALALUDINII</i> .....	 170
7.1 Introduction.....	170
7.2 Materials and Methods.....	172
7.2.1 Enzyme Preparation, Enzyme Activity Measurement and Feed Composition.....	172

	Page
7.2.2 <i>In Vitro</i> Digestion and Measurements.....	174
7.2.3 <i>In Vivo</i> Digestion and Measurements .....	177
7.2.4 <i>M. jalaludinii</i> Phytase Activities in Different Gut Contents of Chicken.....	180
7.2.5 Acid Tolerance of <i>M. jalaludinii</i> .....	181
7.2.6 Statistical Analysis.....	182
7.3 Results.....	182
7.3.1 <i>In Vitro</i> Released Phosphorus.....	182
7.3.2 <i>In Vivo</i> Digestion.....	186
7.3.3 Regression Analysis of <i>In Vivo</i> Digestion Response and <i>In Vitro</i> Released Phosphorus from Corn- soybean Meal Diets by <i>M. jalaludinii</i> Phytase .....	189
7.3.4 <i>M. jalaludinii</i> Phytase Activities in Different Gut Contents of Broiler Chickens.....	191
7.3.5 Acid Tolerance of <i>Mitsuokella jalaludinii</i> .....	191
7.4 Discussion.....	193
 8 EFFICACY OF SUPPLEMENTATION OF <i>M.</i> <i>JALALUDINII</i> CULTURE AND NATUPHOS® PHYTASE ON THE PERFORMANCE AND NUTRIENT UTILISATION OF BROILER CHICKENS FED CORN-SOYBEAN MEAL DIETS.....	198
8.1 Introduction.....	198
8.2 Materials and Methods.....	199
8.2.1 Animals and Rearing Management.....	199
8.2.2 Experiment I.....	200
8.2.3 Experiment II.....	208
8.2.4 Statistical Analysis.....	211
8.3 Results.....	211
8.3.1 Experiment I.....	211
8.3.2 Experiment II.....	227
8.4 Discussion.....	238
 9 GENERAL DISCUSSION AND CONCLUSIONS.....	250
9.1 General Discussion .....	250
9.2 Conclusions.....	265
 BIBLIOGRAPHY.....	267
VITA .....	294



## LIST OF TABLES

Table	Page
1    Phytate phosphorus contents of some feedstuffs commonly used in chickens feed .....	11
2    Some general characteristics of phytase from different sources.....	28
3    Michaelis constants ( <i>K<sub>m</sub></i> ) of phytase .....	29
4    Substrate specificity of some phytases .....	31
5    Effects of some reagents and cations on the activity of phytase .....	32
6    Effectiveness of microbial phytase on the performance and retention of P in broiler chickens fed maize-soybean meal diet.....	35
7    Improvement of phytate hydrolysis and total phosphorus retention of feed ingredients by adding phytase.....	36
8    Phosphorus (P) equivalency of microbial phytase for broiler chickens.....	36
9    Composition of MPS medium (per litre).....	48
10   Composition of MM 10 medium (per litre).....	49
11   Media Used for Biochemical Tests.....	57
12   Ability of 250 bacterial colonies to liberate P from Na-phytate in MM 10 and MPS broth after 24 h incubation .....	61
13   Characteristics of rumen bacterial strains M 1, M 3, M 4, M 7 and M 9 ( <i>Mitsuokella jalaludinii</i> ) and <i>Mitsuokella multiacida</i> type strain ATCC 27723 <sup>T</sup>	66
14   %16S rRNA gene sequence similarity value for type strain M 9 <sup>T</sup> ( <i>Mitsuokella jalaludinii</i> ) and related taxa.....	72
15   Composition of MPYG medium.....	82
16   Media used to study phytase activity of <i>M. jalaludinii</i> .....	88
17   Percentages of Na-phytate hydrolysed by <i>Mitsuokella jalaludinii</i> growing in MPYG medium under aerobic and anaerobic conditions.....	92

	<b>Page</b>
18 Growth of <i>Mitsuokella jalaludinii</i> in MF 1 broth with or without phosphate or Na-phytate .....	94
19 Inorganic phosphorus (P) concentrations of MF 1 medium with or without phosphate or Na-phytate incubated with <i>M. jalaludinii</i> .....	95
20 Effects of KH <sub>2</sub> PO <sub>4</sub> and Na-phytate supplementations to MF 1 medium on the phytase production of <i>M. jalaludinii</i> at different incubation periods.....	97
21 The counts of viable <i>M. jalaludinii</i> grown in different media at 12 h of fermentation.....	99
22 Phytase activities produced by <i>M. jalaludinii</i> in different media at different fermentation periods.....	101
23 Phytase activities and total cell counts (TCC) of <i>M. jalaludinii</i> in media with various glucose concentrations.....	102
24 Effects of phosphate supplementation to RB-SM medium on phytase activity of <i>M. jalaludinii</i> at different fermentation periods.....	104
25 Effects of pH on phytase production at different fermentation periods and total cell count of <i>M. jalaludinii</i> at 12 h of fermentation.....	105
26 Effects of incubation temperatures on phytase production of <i>M. jalaludinii</i> .....	107
27 Effect of various surfactants and polyethylene glycol on phytase production of <i>M. jalaludinii</i> after 12h of fermentation.....	108
28 Effects of different concentrations of rice bran (RB) and soybean milk (SM) on phytase activity and total cell count (TCC) of <i>M. jalaludinii</i> at 12h of fermentation.....	110
29 Purification scheme of phytase from <i>Mitsuokella jalaludinii</i> .....	131
30 Effect of temperature on <i>M. jalaludinii</i> phytase activity.....	134
31 Effect of pH on phytase activity of <i>M. jalaludinii</i> .....	135
32 Effect of pH on the stability of <i>M. jalaludinii</i> phytase activity....	136
33 Substrate specificity of <i>Mitsuokella jalaludinii</i> phytase.....	138

	<b>Page</b>
34 Effects of reagents and metal ions on the phytase activity of <i>M. jalaludinii</i> .....	140
35 Extraction of cell-associated phytase from <i>M. jalaludinii</i> .....	142
36 Pathogenicity of <i>M. jalaludinii</i> inoculated by different routes into mice.....	155
37 Rectal temperatures of mice challenged with <i>M. jalaludinii</i> .....	156
38 Serum SD and GOT activities of mice challenged with <i>M. jalaludinii</i> .....	157
39 Organ weights of mice challenged with <i>M. jalaludinii</i> .....	158
40 Recovery of <i>M. jalaludinii</i> from the blood of inoculated mice.....	159
41 Detection of <i>M. jalaludinii</i> in various organs of mice sacrificed at the end of the experimental period.....	160
42 Rectal temperatures of chickens challenged with <i>M. jalaludinii</i> .....	161
43 Serum glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase activities of chickens challenged with <i>M. jalaludinii</i> .....	162
44 Organ weights of chickens challenged with <i>M. jalaludinii</i> .....	163
45 Body weight gains of chickens challenged with <i>M. jalaludinii</i> .....	164
46 Feed intakes and feed conversion ratio of chickens challenged with <i>M. jalaludinii</i> (21– 42 days of age).....	165
47 Recovery of <i>M. jalaludinii</i> from blood of inoculated chickens.....	166
48 Detection of <i>M. jalaludinii</i> in various organs of inoculated chickens (42 days of age) sacrificed at the end of the experimental period.....	166
49 Composition of experimental diets .....	173
50 Phosphorus released from the feed supplemented with <i>M. jalaludinii</i> cell-bound phytase using Tervilä-Wilo's simulation method.....	183

	Page
51 The difference in released phosphorus between various simulated digestion processes using Tervilä-Wilo's simulation method.....	184
52 Phosphorus released from the feed supplemented with <i>M. Jalaludinii</i> cell-bound phytase using Zyla's simulation method .....	185
53 Effects of supplementation of different levels of <i>M. jalaludinii</i> cell-bound phytase on apparent metabolisable energy (AME) of diets, and digestibilities of dry matter (DM), crude protein (CP) and phosphorus (P) <i>in vivo</i> (42-day-old broilers).....	188
54 The R <sup>2</sup> values of different regression models tested for fitting the results of the <i>in vitro</i> experiments and the results of an <i>in vivo</i> digestion experiment with broiler chickens.....	190
55 Phytase activities in diets and gastrointestinal tracts of 42-day-old chickens fed corn-soybean meal diets containing two levels of available phosphorus (aP) and added with different levels of <i>M. jalaludinii</i> cell-bound phytase.....	192
56 Survival of <i>M. jalaludinii</i> at various pH, as determined by counts of viable bacteria.....	193
57 Composition of basal diets for Experiment I.....	201
58 Composition of basal diets for Experimental II.....	210
59 Body weights and body weight gains of broiler chickens fed corn-soybean meal diets supplemented with different levels of <i>M. jalaludinii</i> phytase (AMJC).....	214
60 Feed intakes and feed conversion ratios of broiler chickens fed corn-soybean meal diets supplemented with different levels of <i>M. jalaludinii</i> phytase (AMJC).....	215
61 Effects of AMJC supplementation on apparent metabolisable energy (AME) and apparent digestibilities of crude protein (CP) and dry matter (DM) of diets in broiler chickens.....	220
62 Effects of supplementation of AMJC on relative retention of calcium (Ca) and total phosphorus (P) in broiler chickens.....	221
63 Effects of AMJC supplementation on intake and excretion of phosphorus in broiler chickens fed corn-soybean diet.....	221

	Page
64 Effects of supplementation of active <i>M. jalaludinii</i> culture (AMJC) on relative retention rates of manganese (Mn), zinc (Zn) and copper (Cu) in broiler chickens fed corn-soybean diets.....	222
65 Effects of supplemental AMJC on the tibia bone ash, phosphorus (P), calcium (Ca), manganese (Mn), zinc (Zn) and copper (Cu) contents in bone ash of tibia of 21-day-old broiler chickens.....	224
66 Effects of supplemental AMJC on the phosphorus (P), calcium (Ca), manganese (Mn), zinc (Zn) and copper (Cu) contents in DM of tibia bone of 21-day-old broiler chickens fed corn-soybean meal diets.....	224
67 Effects of AMJC supplementation on the concentrations of plasma total phosphorus, calcium, manganese and zinc in 21-d-old broiler chickens.....	225
68 Effects of AMJC supplementation on organ weights.....	226
69 Effects of FD-AMJC and Natuphos <sup>®</sup> phytase supplementations on the performance of broiler chickens fed corn-soybean meal diets.....	229
70 Effects of FD-AMJC and Natuphos <sup>®</sup> phytase supplementations on the apparent metabolisable energy (AME) of diets and apparent digestibilities of crude protein (CP) and dry matter (DM) in broiler chickens.....	230
71 Effects of supplementations of FD-AMJC and Natuphos <sup>®</sup> phytase on the relative retention of total phosphorus (P), calcium (Ca), manganese (Mn), zinc (Zn) and copper (Cu) in broiler chickens fed corn-soybean diet.....	233
72 Effects of supplementations of FD-AMJC and Natuphos <sup>®</sup> phytase on intake and excretion of phosphorus in broiler chickens fed corn-soybean meal diet.....	234
73 Effects of FD-AMJC and Natuphos <sup>®</sup> phytase supplementations on the tibia bone ash, P, Ca, Mn and Zn contents in bone ash of tibia of 21-day-old broiler chickens.....	236
74 Effects of FD-AMJC and Natuphos <sup>®</sup> phytase supplementations on the P, Ca, Mn and Zn contents in DM of tibia of 21-day-old broiler chickens.....	237

	<b>Page</b>
75    Effects of FD-AMJC and Natuphos <sup>®</sup> phytase supplementations on the plasma total phosphorus (P), calcium (Ca), manganese (Mn) and zinc (Zn) concentrations in 21-day-old broiler chickens fed corn-soybean meal diets.....	238

## LIST OF FIGURES

Figure		Page
1	The molecular structure of phytic acid (Anderson, 1914).....	13
2	Phytic acid chelate at neutral pH.....	16
3	Procedure for isolation of phytase-producing bacteria from the rumen.....	51
4	Phytase activity in fractionated rumen fluid samples from the rumen of cattle.....	60
5	Scanning electron micrograph of (a) <i>Mitsuokella multiacida</i> ATCC 27723 <sup>T</sup> (= DSM 20544 <sup>T</sup> ) and (b) rumen bacterial strain M 9 ( <i>Mitsuokella jalaludinii</i> ) (bar = 10 µm).....	62
6	Colonies of rumen bacterial strain M 9 ( <i>M. jalaludinii</i> ) grown on PYG agar for 48 h.....	63
7	Growth of rumen bacterial strain M 9 ( <i>M. jalaludinii</i> ) at different temperatures.....	65
8	Growth of <i>Mitsuokella multiacida</i> ATCC 27723 <sup>T</sup> at different temperature. <i>M. multiacida</i> ATCC 27723 <sup>T</sup> could not grow at temperatures of 42, 45 and 47 °C.....	65
9	Full sequence of 16S rRNA gene of type strain M 9 <sup>T</sup> ( <i>Mitsuokella jalaludinii</i> ).....	71
10	Phylogenetic dendrogram of type strain M 9 <sup>T</sup> ( <i>Mitsuokella jalaludinii</i> ) based on 16S rRNA gene sequence data.....	73
11	Growth of <i>M. jalaludinii</i> in MPYG medium under aerobic and anaerobic conditions.....	92
12	Na-phytate concentration in MPYG medium cultured with <i>M. jalaludinii</i> under aerobic and anaerobic conditions.....	93
13	Growth of <i>M. jalaludinii</i> in MF 1 medium with or without phosphate or Na-phytate.....	94
14	Phytase activity of <i>M. jalaludinii</i> cultured in MF 1 broth with or without phosphate or Na-phytate.....	98
15	pH of MF 1 medium with or without phosphate or Na-phytate.....	98

	<b>Page</b>
16 Effects of glucose supplementation to RB-SM medium on phytase production by <i>M. jalaludinii</i> .....	103
17 Effects of phosphate concentrations of RB-SM medium on phytase production of <i>M. jalaludinii</i> .....	104
18 Effects of initial pH values of RB-SM medium on phytase production by <i>M. jalaludinii</i> .....	106
19 Effects of incubation temperatures on phytase production of <i>M. jalaludinii</i> .....	107
20 Phytase-active fractions from the second anion exchange chromatography.....	132
21 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of purified phytase.....	133
22 Effect of temperature on the activity of <i>M. jalaludinii</i> phytase.....	134
23 Effect of pH on phytase activity of <i>M. jalaludinii</i> .....	136
24 Effect of pH on the stability of <i>M. jalaludinii</i> phytase.....	137
25 Effect of substrate concentration ([S]) on phytase reaction velocity (v).....	139
26 Inhibitory effect of phosphate on phytase activity.....	141
27 Procedure to isolate <i>M. jalaludinii</i> from the organs of mice challenged with <i>M. jalaludinii</i> .....	151
28 Spleens of control chickens and chickens intravenously injected with $4.5 \times 10^{10}$ viable cells of <i>M. jalaludinii</i> .....	163
29 A stainless steel funnel with a stainless plunger used for precision feeding in broiler chicken.....	178
30 Precision feeding in broiler chicken.....	179
31 Effects of phytase concentrations from <i>M. jalaludinii</i> on the release of phosphorus from feed in diet 2 (0.24% aP) determined using <i>in vitro</i> simulation procedure of Tervilä-Wilo <i>et al.</i> (1996).....	184



	<b>Page</b>
32      Effects of phytase concentrations from <i>M. jalaludinii</i> on the release of phosphorus from feeds in diet 1 (0.46% aP) determined using <i>in vitro</i> simulation procedure of Tervilä-Wilo <i>et al.</i> (1996).....	185
33      Effects of phytase concentrations from <i>M. jalaludinii</i> on the release of phosphorus from feeds in diet 1 (0.46% aP) and diet 2 (0.24% aP) determined using <i>in vitro</i> simulation procedure of Zyla <i>et al.</i> (1995a).....	186
34      Apparent metabolisable energy (AME) of corn-soybean diets for broiler chickens supplemented with <i>M. jalaludinii</i> cell-bound phytase. The polynomial model was used for the regression analysis.....	187